Disease suppression by the ectomycorrhizal fungus *Paxillus involutus:* contribution of oxalic acid

LUC C. DUCHESNE[1]
Department of Botany, University of Guelph, Guelph, Ont., Canada N1G 2W1

BRIAN E. ELLIS[2]
Department of Chemistry and Biochemistry and Department of Botany, University of Guelph, Guelph, Ont., Canada N1G 2W1

AND

R. L. PETERSON
Department of Botany, University of Guelph, Guelph, Ont., Canada N1G 2W1

Received January 1, 1989

DUCHESNE, L. C., ELLIS, B. E., and PETERSON, R. L. 1989. Disease suppression by the ectomycorrhizal fungus *Paxillus involutus*: contribution of oxalic acid. Can. J. Bot. **67**: 2726–2730.

Seedlings of *Pinus resinosa* Ait. grown in test tubes were inoculated with the ectomycorrhizal fungus *Paxillus involutus* Fr. Oxalic acid was identified as one of the ethanol-soluble fungistatic and (or) fungitoxic components of the rhizosphere after fractionation by high performance liquid chromatography, paper chromatography, and gel filtration. Simultaneous inoculation of *P. resinosa* seedlings with authentic oxalic acid and a spore suspension of *Fusarium oxysporum* f.sp. *pini* protected the seedlings against *Fusarium* root rot and decreased the sporulation of *F. oxysporum* in the rhizosphere when compared with controls lacking oxalic acid. Quantitation of oxalic acid showed a five fold increase in production by *Pax. involutus* in tubes containing *P. resinosa* seedlings when compared with tubes lacking seedlings. The synthesis of oxalic acid by *Pax. involutus* is, therefore, stimulated by *P. resinosa* root exudate.

DUCHESNE, L. C., ELLIS, B. E., et PETERSON, R. L. 1989. Disease suppression by the ectomycorrhizal fungus *Paxillus involutus*: contribution of oxalic acid. Can. J. Bot. **67** : 2726–2730.

On a inoculé des semis de *Pinus resinosa* Ait. en éprouvettes avec le champignon ectomycorhizateur *Paxillus involutus* Fr. L'acide oxalique était un des composés fongitoxiques et (ou) fongistatiques contenus dans les extraits à l'éthanol de la rhizosphère identifié au moyen de la chromatographie en phase liquide à haute performance, la chromatographie sur papier et la chromatographie sur gel. Le traitement de semis avec une solution d'acide oxalique et une suspension de spores de *Fusarium oxysporum* f.sp. *pini* a réduit la mortalité des semis et la sporulation du pathogène dans la rhizosphère des semis traités à l'acide oxalique en comparaison à celle des semis inoculés avec le pathogène mais sans acide oxalique. La quantification de l'acide oxalique a démontré cinq fois plus d'acide oxalique dans le cas de semis inoculés avec *Pax. involutus* que dans le cas d'éprouvettes inoculées avec *Pax. involutus* seulement. La synthèse de l'acide oxalique par ce champignon est donc stimulée par les exsudats racinaires de *P. resinosa*.

Introduction

Information concerning the synthesis and accumulation of antimicrobial compounds by ectomycorrhizal fungi is scanty, although over 90 ectomycorrhizal fungi have been reported to synthesize such substances (Marx 1973). The synthesis of polyacetylene antibiotics by the ectomycorrhizal fungus *Leucopaxillus cerealis* var. *piceina* Peck. *in vitro* and *in situ* was reported by Marx (1969*a*, 1969*b*) and Marx and Davey (1969). Otherwise, few fungi species have been chemically investigated.

Only a limited number of investigations have addressed the synthesis of antibiotics by ectomycorrhizal fungi *in vitro* and *in situ.* It is difficult, therefore, to generalize about the role of antibiosis by ectomycorrhizal fungi in natural ecosystems. A better understanding of antibiotic biosynthesis will be essential if ectomycorrhizal fungi, or antifungal compounds synthesized by ectomycorrhizal fungi (Garrido *et al.* 1982), are to be employed as biological pesticides against root pathogens.

Assessment of the potential impact of antibiotic biosynthesis by ectomycorrhizal fungi has been conducted commonly by challenging a test mycorrhizal fungus with a pathogenic fungus on nutrient agar slabs. Recent investigations have demonstrated, however, that this technique may not always be appropriate because antibiosis can vary with the nutrient content of the growth medium (Whipps 1987) or may be modified when ectomycorrhizal fungi interact with root exudates (Duchesne *et al.* 1988*b*).

The presence of antifungal compounds produced by *Paxillus involutus* is associated with a significant increase in the resistance of *Pinus resinosa* seedlings to infection by the root pathogenic fungus *Fusarium oxysporum* Schlecht. emend Snyd. & Hans f.sp. *pini* (Duchesne *et al.* 1988*a*). The identification of the *Pax. involutus* metabolites, which may be involved in disease suppression by this fungus, is a crucial step in clarifying this phenomenon and in assessing the feasibility of field application of *Pax. involutus* as a biological deterrent of root diseases. This paper reports the isolation and identification of oxalic acid as a nonvolatile fungitoxic and (or) fungistatic chemical synthesized by the ectomycorrhizal fungus *Pax. involutus* in the rhizosphere of *P. resinosa* seedlings.

Materials and methods

Seedling and fungal cultivation

Most aspects of seedling cultivation have been described elsewhere

[1]Author to whom all correspondence should be addressed at Department of Botany, University of Toronto in Mississauga, Mississauga, Ont., Canada L5L 1C6.

[2]Present address: Department of Plant Science, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

(Duchesne *et al.* 1988*a*). Briefly, seeds of *P. resinosa* were surface sterilized using 30% H_2O_2 for 45 min. The seeds were washed with 1 L autoclaved distilled water and subsequently left to germinate in Petri dishes lined with wet Whatman filter paper. Ten to 12 days after seed sterilization, the seedlings were transferred to test tubes as described in Duchesne *et al.* (1988*a*). All aspects of cultivation of the isolates of *Pax. involutus* and *F. oxysporum* have been described elsewhere (Duchesne *et al.* 1988*a*).

Inoculation with Pax. involutus

Seedlings in test tubes were inoculated with *Pax. involutus* and incubated for 2 weeks (Duchesne *et al.* 1988*a*). Controls consisted of seedlings inoculated with sterile plugs of modified Melin Norkrans medium (MMN). After removing the seedlings, the contents of the tubes were extracted using 50% ethanol (Duchesne *et al.* 1988*a*). The crude extracts from the rhizosphere of one seedling are designated as 1 seedling extractive equivalent (1 SEE). Fractionation of the crude extracts was conducted using HPLC analysis, gel filtration, and paper chromatography.

HPLC analysis of crude extracts

The crude extracts were filtered through 0.45 μm Millipore nylon filters, and aliquots (2.5 SEE) were fractionated by reverse phase HPLC (Alltech Econosphere C-18, 5 μm pore size, 250 × 4.6 mm). The elution program was executed by a Gilson 714 HPLC control system at a rate of 1 mL/min: water for 5 min, a linear gradient from 100% water to 100% acetonitrile over a period of 30 min, and finally 100% acetonitrile for 10 min. All solvents were glass distilled and filtered.

Comparison was made of the fungitoxic activity of individual fractions derived from the rhizosphere of seedlings inoculated with *Pax. involutus* with those from the rhizosphere of control seedlings. The eluate was monitored at a wavelength of 210 nm and collected as 1-mL fractions, which were bioassayed on *F. oxysporum* microconidia germination (Duchesne *et al.* 1988*a*) to determine the presence of fungitoxic substances. Bioassay of each of these fractions was performed on duplicate aliquots containing approximately 1 SEE of the extractives for a given fraction. This experiment was repeated three times. The results were transformed (y = arcsin $X^{0.5}$, before being compared using Student's t-test at $P < 0.01$ (Sokal and Rohlf 1981).

Gel filtration

Further purification of the major fungitoxic peak (HPLC fractions 3–5 min) was carried out using gel filtration on LH-20 Sephadex (Pharmacia). Fractions 3–5 were collected from the injection of 20 SEE, pooled, evaporated to dryness, taken up in 1 mL distilled water, and loaded on a 1 × 30 cm LH-20 column bed. The column was then eluted with 200 mL distilled water, followed with 100 mL methanol each at a rate of 3 mL/min using a LKB2132 Microperpex peristaltic pump. The eluate was collected in 6-mL fractions and bioassayed using 100-μL and 1000-μL aliquots. This experiment was repeated twice. All detectable fungitoxic activity was eluted with water between 36 and 66 mL. These fractions from approximately 20 SEE of crude extracts were pooled, evaporated to dryness, and taken up in 2.0 mL water.

Paper chromatography

Further purification of the LH-20 fungitoxic material was carried out by descending paper chromatography on Whatman 3MM chromatography paper eluted with 1-propanol – 1 M ammonium hydroxide (3:1, v/v). After drying overnight, strips (1.5 × 17 cm) were cut from the sides of the chromatogram. These paper strips were cut in 1.5 × 1.0 cm pieces and placed on microscope slides in Petri dishes lined with wet filter paper. A *F. oxysporum* spore suspension (100 μL; 10^5 spores in 4% ethanol) was placed on top of each piece of paper, incubated for 14 h before fixation with lactophenol blue, and evaluated for microconidia germination. The bioassay detected a single fungitoxic band. This band on the remainder of the chromatogram, which had been stored at −10°C in the dark, was extracted using 50% ethanol at 4°C in the dark. The ethanol was evaporated to dryness and the dry residue was taken up in 1.5 mL water (~100 μL/SEE).

This partly purified material was analyzed on Whatman 3MM chromatography paper using *l*-propanal – 1 M ammonium hydroxide (7:3, v/v), ethanol – 1 M ammonium hydroxide (19:1, v/v), and *n*-butanol – formic acid – water (4:1:5, v/v/v) and on Whatman silica gel 60A K6F TLC plates using methanol – 5 M ammonium hydroxide (4:1, v/v). The developed plates were tested for reactivity with iodine vapour, diazotized *p*-nitroaniline (Van Sumere *et al.* 1965), and bromophenol blue (0.3%) plus methyl red (0.1%) in 95% ethanol (Ting and Dugger 1965).

Protective effect of oxalic acid

The ED_{50} value of authentic oxalic acid on *F. oxysporum* microconidia germination was determined by incubating spores of this fungus with different quantities of the chemical (20–3000 μg/100 μL). The value was found to be 50 μg/100 μL under our assay conditions. Eighty seedlings in test tubes were inoculated with *F. oxysporum* (10^5 spores) 1 day after transfer to the tubes, to each of which 1 mL of a filter-sterilized aqueous solution of oxalic acid (125 μg/mL; 125 μg = 2.5 × ED_{50}) was also added. This quantity of oxalic acid was used because the total fungitoxic activity of the rhizosphere of one seedling inoculated with *Pax. involutus* is equivalent to 2.50 ED_{50} value (Duchesne *et al.* 1988*a*, 1988*b*). Controls consisted of 40 seedlings inoculated with 1 mL filter-sterilized distilled water. Survival of the seedlings and sporulation of *F. oxysporum* in the tubes were assessed 2 weeks after inoculation, as described elsewhere (Duchesne *et al.* 1988*a*). This experiment was repeated three times. Statistical analysis of seedling survival was carried out using the G-statistics at $P <$ 0.01, whereas statistical analysis of *F. oxysporum* sporulation was carried out using the t-test at $P < 0.01$ (Sokal and Rohlf 1981).

Quantitation of oxalic acid

The oxalic acid content of rhizosphere extracts of seedlings inoculated with *Pax. involutus* was compared with that of seedlings alone and with that of *Pax. involutus* from test tubes without seedlings. Inoculation of the seedlings with *Pax. involutus* or discs of sterile MMN medium was as in Duchesne *et al.* (1988*a*) and inoculation of tubes without seedlings as in Duchesne *et al.* (1988*b*). Rhizosphere extracts were prepared 2 weeks after inoculation of the seedlings, as described previously.

Quantitation of oxalic acid was performed using the HPLC procedure developed by Lapeyrie *et al.* (1987). Aqueous samples (5 SEE) were adsorbed onto QAE-Sephadex anion exchange resin and eluted with HCl. The HCl fraction was collected, evaporated to dryness under reduced pressure, and left overnight in a desiccator with KOH pellets. The samples were then dissolved in 0.05 M H_2SO_4 containing 10 mM succinic acid (as internal standard) and filtered through a Gelman Acro LCS3S 0.45-μm filter assembly. Aliquots containing 0.05 SEE were analyzed using an Aminex HPX87H column (300 × 7.6 mm) (BioRad). The samples were eluted with 0.05 M H_2SO_4 at a flow rate of 0.6 mL/min and the eluate was monitored at 210 nm. The area of the oxalic acid peak in the samples was proportional to that of authentic oxalic acid injected over the range of 0.05–1 μg. The recovery of authentic oxalic acid through this procedure (85%) was similar to that of Redgwell (1980). This experiment was repeated three times and statistical analysis was carried out using the Mann–Whitney U-test at $P < 0.01$ (Sokal and Rohlf 1981).

Results

HPLC analysis of crude extracts

HPLC analysis of the rhizosphere crude extracts indicated the presence of newly produced compounds or compounds present in greater quantities in the rhizosphere of *P. resinosa* seedlings after the inoculation with *Pax. involutus*. Examples of these compounds include peaks eluting at 16, 21, 39, and 41 min (Fig. 1). Preliminary experiments revealed that most of the fungitoxic activity of the crude extracts from the rhizosphere of seedlings inoculated with *Pax. involutus* was eluted within the first 20 min of the elution program; therefore, no

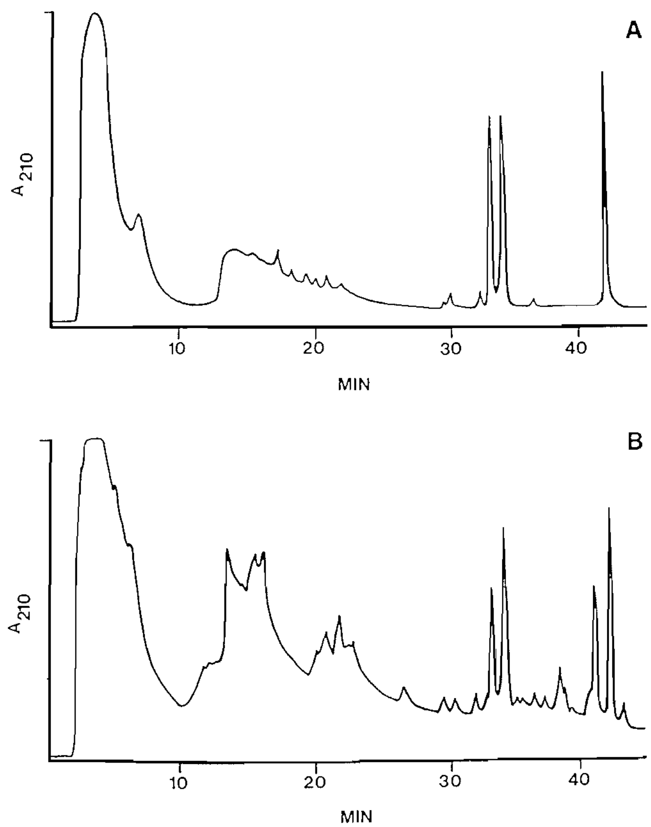

FIG. 1. HPLC analysis of crude extracts from the rhizosphere of *Pinus resinosa* seedlings. (A) Control seedlings. (B) Seedlings inoculated with *Paxillus involutus*.

TABLE 1. Fungitoxic activity of crude extracts from the rhizosphere of *Pinus resinosa* seedlings inoculated with *Paxillus involutus* after HPLC fractionation

	% *Fusarium* germination	
Fraction	*Pax. involutus*	Control
1	92	99
2	89	101
3	22*a*	98*a*
4	0*a*	98*a*
5	42*a*	104*a*
6	82	100
7	90	96
8	90	96
9	95	100
10	91	93
11	41*a*	91*a*
12	86	97
13	89	98
14	82	97
15	47*a*	97*a*
16	97	100
17	91	94
18	86	93
19	92	93
20	94	97
21−45	100	100

NOTE: The eluent was collected in 1-mL fractions that were evaporated to dryness and bioassayed for fungitoxic activity on *Fusarium oxysporum* microconidia germination. Fractions 1−20 were assayed separately, whereas fractions 21−45 were pooled. Within lines, values followed by a letter are significantly different at $P < 0.01$; the values are based on an average of three repetitions.

further attempt was made to isolate fungitoxic chemicals from fractions 21−45. The fungitoxic activity from inoculated seedlings was eluted in three major bioactive fractions at 3−5, 11, and 15 min, whereas no detectable fungitoxic activity of the rhizosphere extracts of the control seedlings was observed (Table 1). Characterization of the peaks eluting at 11 and 15 min was not successful.

Further purification of HPLC fractions 3−5 using LH-20 gel filtration indicated the presence of a single fungitoxic substance that comigrated with authentic oxalic acid on chromatography paper and silica gel plates. As with authentic oxalic acid, the purified material did not react with iodine vapor and diazotized p-nitroaniline, whereas it tested positive with the bromophenol blue − methyl red reagent to indicate an acidic property.

Furthermore, analysis of crude rhizosphere extracts directly by paper chromatography (*l*-propanol − 1 M ammonium hydroxide (7:3, v/v)) or by anion exchange chromatography according to Redgwell (1980) yielded a major fungitoxic compound with the chromatographic (TLC, HPLC) and test reagent properties of authentic oxalic acid.

Protective effect of oxalic acid

The treatment of *P. resinosa* seedlings with authentic oxalic acid had a protective effect against *Fusarium* root rot, since the seedling survival rate (61%) was significantly greater than that of control seedlings (23%) (Fig. 2; Table 2). The protective effect of oxalic acid was also associated with a 43% suppression of the sporulation of *F. oxysporum* in the rhizosphere of the seedlings (Table 2).

Quantitation of oxalic acid

The inoculation of *P. resinosa* seedlings with *Pax. involutus* resulted in greater concentrations of oxalic acid in the rhizosphere of these seedlings compared with seedlings inoculated with plugs of sterile MMN medium (Table 3). The presence of *P. resinosa* seedlings in the tubes stimulated the synthesis of oxalic acid by *Pax. involutus* since the oxalic acid concentration in tubes containing *Pax. involutus* and *P. resinosa* seedlings was fivefold greater than the concentration of oxalic acid in tubes inoculated with *Pax. involutus* but without seedlings (Table 3).

Discussion

The synthesis of oxalic acid has been reported in numerous fungal species (Hodgkinson 1977). It has been postulated that oxalic acid acts as a weathering agent in soils (Cromack *et al.* 1979; Entry *et al.* 1987), and its biosynthesis by *Pax. involutus* has been postulated as reducing calcium toxicity in calcicole soils (Lapeyrie and Bruchet 1986). The results from this investigation suggest that oxalic acid may also participate in disease protection by *Pax. involutus*.

In the present study, oxalic acid was isolated as a fungitoxic and (or) fungistatic component from the rhizosphere of *P. resinosa* seedlings inoculated with *Pax. involutus*. The concentration of oxalic acid in the rhizosphere of the seedlings is low, however, compared with the overall fungitoxic activity of the extracts. In previous experiments, it was observed that the

FIG. 2. (A) Seedlings of *Pinus resinosa* inoculated with authentic oxalic acid (125 μg/seedling; 21 μg/mL) and *Fusarium oxysporum*. (B) Seedlings inoculated with autoclaved distilled water and *F. oxysporum*.

TABLE 2. Protective effect of oxalic acid against *Fusarium* root rot of *Pinus resinosa*

Treatment	Seedling survival (%)	Sporulation of *F. oxysporum* (10^6 spores/tube)
Oxalic acid	61.4*a*	2.75*a*
Control	22.6*b*	4.83*b*

NOTE: Within columns, values followed by a different letter are significantly different at $P < 0.01$; the values are based on an average of three repetitions. Seedlings were treated with an aqueous solution of authentic oxalic acid (125 μg/seedling).

TABLE 3. Concentration of oxalic acid in the rhizosphere of *Pinus resinosa* seedlings inoculated with *Paxillus involutus*

Origin of extractives	Oxalic acid (μg/tube)
Control seedlings	<0.33*a*
Pax. involutus in tubes	
Nutrient solution only	1.79*b*
With *P. resinosa* seedlings	8.33*c*

NOTE: Values followed by a different letter are significantly different at $P < 0.01$; the values are based on an average of three repetitions.

rhizosphere of one *P. resinosa* seedling inoculated with *Pax. involutus* contains ~2.5 ED_{50} values (Duchesne *et al.* 1988*a*, 1988*b*). The quantities of oxalic acid observed in the present experiment, however, can only account for 0.20 ED_{50} value (8.83 μg/SEE), since the ED_{50} value of authentic oxalic acid in the same assay conditions is 50 μg. Three hypotheses could account for this discrepancy. First, other fungitoxic and (or) fungistatic chemicals of the rhizosphere may have comigrated with oxalic acid in all of the solvent systems used in this experiment, thus yielding a high apparent bioactivity. If this were the case, such substances must be chemically similar to oxalic acid, since all the methods used in this paper led to the isolation of oxalic acid as the only fungitoxic substance in HPLC fractions 3–5. Second, other chemicals present in the rhizosphere may enhance the biological activity of oxalic acid, thus lowering its apparent ED_{50} value *in situ*. Fractions 11 and 15 from HPLC analysis and other chemicals not found fungitoxic may be involved in enhancing the effect of oxalic acid. Third, chemicals present in the rhizosphere, particularly metals capable of chelating with oxalic acid, may form strong complexes with oxalic acid and lower its effective concentration in the rhizosphere.

Another reason why the contribution of oxalic acid to disease suppression by *Pax. involutus* remains uncertain is that, although the results presented in this paper suggest that oxalic acid may be involved in this phenomenon, it has yet to be demonstrated that oxalic acid is present in the rhizosphere of the seedlings at a time that coincides with the onset of seedling protection (Duchesne *et al.* 1989*b*). Further investigation is needed to address this question.

The mechanisms involved in the stimulation of oxalic acid biosynthesis by *P. resinosa* root exudate have not been determined. The work of Lapeyrie *et al.* (1987) showed that the synthesis of oxalic acid by *Pax. involutus* can be influenced *in vitro* by the content of nitrogen, calcium, and carbonates in the culture medium. It is possible that these factors are also

involved in the stimulation of antibiosis by *Pax. involutus* in the rhizosphere of *P. resinosa* seedlings. An understanding of these factors may help manipulate *Pax. involutus*, or edaphic conditions, for enhanced disease suppression. Our results do not indicate, however, whether the stimulation of oxalate synthesis in the rhizosphere of the seedlings is the result of enhanced fungal growth or of differential gene expression in *Pax. involutus*.

The treatment of seedlings with authentic oxalic acid led to a 43% decrease in the sporulation of the pathogen in the rhizosphere of the seedlings. In other experiments (Duchesne *et al.* 1988*a*, 1988*b*), inoculation of seedlings or root exudate with *Pax. involutus* led to 80% depression in the sporulation of the pathogen in the rhizosphere of the seedlings. Since the quantities of oxalic acid used in this experiment corresponded to the overall fungitoxic activity of the rhizosphere of seedlings inoculated with *Pax. involutus*, one would expect pathogen suppression to be similar whether seedlings were treated with authentic oxalic acid or inoculated with *Pax. involutus*. These results suggest that mechanisms other than, or in addition to, the synthesis of antifungal compounds participate in disease suppression by *Pax. involutus*. Since pathogen reduction was identical when either root exudate or seedlings were inoculated with *Pax. involutus* and *F. oxysporum* (Duchesne et al. 1988*a*, 1988*b*), it is likely that these other possible suppressive mechanisms are generated by the fungus rather than by the plant.

The present data provides support for the contention that the study of the synthesis of antimicrobial compounds by ectomycorrhizal fungi can contribute to the development of means of control for root pathogens in forest nurseries (Duchesne 1988; Duchesne *et al.* 1989*a*). The use of oxalic acid as a pesticide in forest nurseries does not appear to have been examined, but more studies are required before field application of this chemical could be envisioned. In particular, the protective effect of oxalic acid in soils must be ascertained against a number of pathogens, and its environmental effects, including its long-term impact on seedling growth, have to be assessed.

Acknowledgements

The authors thank S. E. Campbell, G. N. Hebb, K. Schooley, and T. Wilton for technical assistance and valuable suggestions; B. Kelley of the Canadian Forestry Service for providing seeds of *P. resinosa*, and Dr. J. A. Fortin, Dr. G. Hofstra, and P. Williams for the gift of fungal isolates. Financial support by the Natural Sciences and Engineering Research Council of Canada and the Quebec Ministry of Education (Fonds pour la formation de chercheurs et l'aide à la recherche) is also acknowledged.

CROMACK, K., SOLLINS, P., GRAUSTEIN, W. C., SPEIDEL, K., TODD, A. W., SPYCHER, G., LI, C. Y., and TODD, R. L. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. Soil Biol. Biochem. **11**: 463–468.

DUCHESNE, L. C. 1988. Biochemical mechanisms of disease suppression by the ectomycorrhizal fungus *Paxillus involutus*. Ph.D. thesis, University of Guelph, Guelph, Ont.

DUCHESNE, L. C., PETERSON, R. L., and ELLIS, B. E. 1988*a*. Interaction between the ectomycorrhizal fungus *Paxillus involutus* and *Pinus resinosa* induces resistance to *Fusarium oxysporum*. Can. J. Bot. **66**: 558–562.

——— 1988*b*. Pine root exudate stimulates antibiotic synthesis by the ectomycorrhizal fungus *Paxillus involutus*. New Phytol. **108**: 470–476.

——— 1989*a*. The future of ectomycorrhizal fungi as biological control agents. Phytoprotection, **70**: 51–58.

——— 1989*b*. The time course of disease suppression and antibiosis by the ectomycorrhizal fungus *Paxillus involutus*. New Phytol. **111**: 693–698.

ENTRY, J. A., ROSE, C. L., CROMACK, K., GRIFFITHS, R. P., and CALDWELL, B.A. 1987. The influence of ectomycorrhizal mats on chemistry of a coniferous soil. *In* Mycorrhizae in the next decade: practical applications and research priorities. *Edited by* D. M. Sylvia, L. L. Hung, and J. H. Graham. Proceedings of the 7th North American Conference on Mycorrhizae, Institute of Food and Agricultural Sciences, University of Florida, Gainsville, FL, May 3–8, 1987. p. 93. (Abstr.)

GARRIDO, N., BECERRA, J., MARTICORENA, C., OEHRENS, E., SILVA, M., and HORAK, E. 1982. Antibiotic properties of ectomycorrhizal fungi and saprophytic fungi growing on *Pinus radiata* D. Don. Mycopathologia, **77**: 93–98.

HODGKINSON, A. 1977. Oxalic acid in biology and medicine. Academic Press, New York.

LAPEYRIE, F., and BRUCHET, G. 1986. Calcium accumulation by two strains, calcicole and calcifuge, of the mycorrhizal fungus *Paxillus involutus*. New Phytol. **103**: 133–141.

LAPEYRIE, F., CHILVERS, G. A., and BHEM, C. A. 1987. Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus* Batsch. ex. Fr) Fr. New Phytol. **106**: 139–146.

MARX, D. H. 1969*a*. The influence of ectotrophic mycorrhizal fungi on the resistance to pathogenic infections. I. Antagonism of mycorrhizal fungi to pathogenic fungi and soil bacteria. Phytopathology, **59**: 153–163.

——— 1969*b*. The influence of ectotrophic mycorrhizal fungi on the resistance to pathogenic infections. II. Production, identification, and biological activity of antibiotics produced by *Leucopaxillus cerealis* var. *piceina*. Phytopathology, **59**: 411–417.

——— 1973. Mycorrhizae and feeder root diseases. *In* Ectomycorrhizae: their ecology and physiology. *Edited by* G. C. Marks and T. T. Kozlowski. Academic Press, New York. pp. 351–382.

MARX, D. H., and DAVEY, L. B. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. III. Resistance of aseptically formed mycorrhizae to infection by *Phytophthora cinnamomi*. Phytopathology, **59**: 549–558.

REDGWELL, R. J. 1980. Fractionation of plant extracts using ion-exchange sephadex. Anal. Biochem. **107**: 44–50.

SOKAL, R. R., and ROHLF, F. J. 1981. Biometry. 2nd ed. W. H. Freeman and Co., San Francisco.

TING, I. P., and DUGGER, W. M., JR. 1965. Separation and detection of organic acids on silica gel. Anal. Biochem. **12**: 571–578.

VAN SUMERE, C. F., WOLF, G., TEUCHY, H., and KINT, J. 1965. A new thin-layer method for phenolic substances and phenolics. J. Chromatogr. **20**: 48–60.

WHIPPS, J. M. 1987. Effect of medium on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. New Phytol. **107**: 127–142.